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The International Isocyanate Institute, Inc.

by

Department of Public Health

Tokyo Women's Medical College

Preliminary Study on Skin Sensitization Caused by MDI solutions

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FROM DR. P. J. POPPER, INTERNATIONAL ISOCYANATE INSTITUTE

DATE 15/12/80

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Piperno
Goldberg

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INTRODUCTION

Diphenylmethane diisocyanate (MDI) is one of the isocyanates that has been in increasing industrial demand in recent years. Since it has a higher boiling point and is therefore less volatile than tolylene diisocyanate (TDI), it has been considered to be of low toxicity. Concerning dermatological problems, only several cases of MDI dermatoses, including contact dermatitis caused by crude MDI and hydrogenated MDI have been reported.[1)2)3)] But under certain conditions, such as using MDI at an elevated temperature[4)] or with organic solvents and gases, the invasion of MDI into the body is probably made easier, so that asthma-like symptoms or hypersensitivity pneumonitis occur, as with TDI.[5)6)7)]

In the case of skin sensitization, percutaneous absorption of the sensitizing substance must be considered. The solvent is one of the important factors affecting percutaneous absorption. When a sensitizer is applied with a solvent that enhances cutaneous permeability, its skin-sensitizing ability is naturally increased.[8)]

As MDI is usually utilized with organic solvents by industrial firms, it may be potentially hazardous for skin sensitization. Consequently, it was considered necessary to study the effects of solvents on skin sensitization with MDI.

OBJECTIVES

The purpose of this report was to study the effects of several industrial solvents, which are frequently utilized with MDI, on skin sensitization caused by MDI in mice. The skin-sensitizing abilities of various MDI solutions were evaluated by the modified ear-flank test method.[9] For purposes of comparison with the MDI results, TDI and two other isocyanates were also tested with several solvents in the same manner.

SUMMARY

The effects of solvents on skin sensitization caused by MDI were studied with the modified ear-flank test method in BALB/c mice.

Skin-sensitizing ability was compared in six MDI solutions. Except for olive oil, the other organic solvents chosen here are now frequently utilized by industry to dissolve MDI and its products. Compared with olive oil, the solutions of ethyl acetate, acetone, dichloromethane and toluene had strong sensitizing power. Among the four, there were almost no significant differences.

However, in the case of dimethylformamide (DMF), its primary irritation was very weak, and therefore no allergic reaction was induced upon challenge. In order to clarify this result, three other isocyanates in olive oil and DMF solutions were chosen to compare their skin-sensitizing ability. Dissolved in DMF, TDI, an aromatic isocyanate, as is MDI, failed to sensitize mice. But, HDI (an aliphatic isocyanate) and IPDI (an alicyclic isocyanate) produced skin sensitization in DMF solution, though weaker than that in olive oil.

The reason of the unusual results of aprotic solvents, e.g., DMF, were clarified by separate experiments[16] to be due to the faster rate of moisture absorption of the solvents.

EXPERIMENTAL RESULTS

1. Test animals

BALB/cAnNCrj mice supplied by Charles River Japan, Inc.

Sex: male

Age: 10-11 weeks old

Number: 166 in total, 10 for each MDI solution (ethyl acetate, acetone, dichloromethane, toluene, dimethylformamide, ethyl acetate : olive oil = 1 : 1, acetone : olive oil = 1 : 1 and olive oil suspension) and six for the dimethylsulfoxide solution

50 in total, five for each TDI solution (ethyl acetate, acetone, dichloromethane, toluene and dimethylformamide)

30 in total, three for each isophorone diisocyanate (IPDI) solution (ethyl acetate, acetone, ethyl acetate : olive oil = 1 : 1, acetone : olive oil = 1 : 1 and olive oil)

40 in total, five for each hexamethylene diisocyanate (HDI) and IPDI solution (olive oil and dimethylformamide)

2. Test methods

2-1. Preparation of isocyanate solutions:

The four isocyanates were separately dissolved in each solvent to make sensitizing and challenging solutions. The solutions were freshly prepared each time when they were applied to the mice. The isocyanates and solvents used in this report were as follows:

Isocyanates

MDI : Pure-MDI, provided by Takeda Yakuhin-Kogyo Co. Ltd.

TDI : Tolylene-2,4-diisocyanate, 1st-grade, Wako Pure Chemical Industries,Ltd.

IPDI : provided by Mitsubishi Chemical Ind.Ltd.

HDI : provided by Mitsubishi Chemical Ind.Ltd.

Solvents

Ethyl acetate : Special grade, Kanto Chemical Co.,Inc.

Acetone : Special grade, Kanto Chemical Co.,Inc.

Dichloromethane : Special grade, Kanto Chemical Co.,Inc.

Toluene : Special grade, Kanto Chemical Co.,Inc.

Dimethylformamide : Special grade, Nakarai Chemicals,Ltd.

Dimethylsulfoxide : Special grade, Nakarai Chemicals,Ltd.

Olive oil : Dainippon Seiyaku Co. Ltd.

2-2. Gauging apparatus for ear thickness:

Dial thickness gauge (Type G-1) manufactured by Ozaki Seisakusho Co. Ltd.

Gauging area : 5 mm in diameter

Gauging pressure : 30 g

2-3. Sensitization:

For sensitization, 25% solution of each solvent was used for MDI and 5% for the other isocyanates. With a 1 ml syringe, 3/100 ml of each sensitizing solution was applied in drop form onto the area (ca. 1 cm^2) on the back of mice from which the hair was previously depilated by hand. This application was conducted once a day for five consecutive days. The control mice received equivalent amount of the solvent only.

2-4. Challenge and determination of ear thickness:

After five applications of the sensitizing solution or the solvent alone, all mice were left untreated for three days. The ear thickness was gauged on the fourth day subsequent to the last sensitizing application. Each mouse (including the controls) was then challenged with the same kind of isocyanate and solvent at a lower concentration (1%, 0.3% or 0.1%) than that used for its previous sensitization. The solu-

tion was painted on both sides of the ear using a drawing brush. The ear thickness was measured again at three, 24 and 48 hr postchallenge.

3. Test results

3-1. Observation on MDI sensitization:

During the sensitizing application, the six solutions of 25% MDI -- dichloromethane, toluene, ethyl acetate, acetone and the mixture of the two latters with olive oil-- and olive oil suspension of 25% MDI produced primary irritant dermatitis. Signs such as reddening, swelling, erosion and crusting appeared at the site of the sensitization.

However, the solution of 25% MDI in dimethylformamide (DMF) caused only tiny erosions and depilation at the site. During the sensitizing application, some whitish substance was observed there. With other solutions, no visible matter appeared at the site of sensitization. On the 10th day of the experiment, the site where MDI-DMF solution had been applied did not appear much different from the same site on controls. (Figs. 1,2,3)

3-2. Comparison of ear thickness in mice sensitized with various solutions of MDI:

Fig.4 shows the comparison of increase rate in ear thickness in mice sensitized with MDI in nine kinds of solvents.

The thickness of the ear was determined for both ears at 48 hr postchallenge and the results are expressed here as the increased percentage of the mean value of 10 mice. The significance of the difference between the mean values for the sensitized and the control mice was evaluated using the Student's t test.

When each mouse was challenged with 1% MDI in each solvent, all the sensitized mice, except for the ones sensitized with DMF or DMSO solution, showed a significant increase in ear thickness. The solutions of ethyl acetate, acetone, dichloromethane and toluene showed a high rate

of increase. There was no significant difference among the four systems, although there was a slight difference between acetone and toluene solutions. As a matter of fact, the olive oil suspension showed a small increase. The two solutions of mixed solvents gave results in between the aforementioned four solutions and the olive oil suspension.

As for the DMF solution, the results indicated that it had failed to sensitize mice. Since DMF is a kind of aprotic polar solvent, it was also attempted to use dimethylsulfoxide (DMSO), a similar solvent with MDI to sensitize mice. As shown in Fig.4, the results were the same as with the DMF solution.

3-3. Comparison of ear thickness in mice sensitized with TDI in four different solvents:

For the purpose of comparison with the results of the MDI solutions, TDI dissolved in four different solvents was tested by the same method. For sensitization and challenge, 5% and 0.3% solutions were used, respectively. The results are shown in Fig.5. The results of TDI in olive oil are not shown here, because challenging with 0.3% solution has not yet been performed. But even when challenged with 0.5% solution, there was no significant increase in ear thickness. Therefore, the four solvents used in Fig.5 appear to have strong sensitizing power as compared to olive oil.

The increase rate with toluene solution was markedly high, but it must be noted that the rate of the control was also considerably high.

3-4. Comparison of ear thickness in mice sensitized with IPDI in five different solvents:

The five different solutions of IPDI were also compared for skin sensitization ability. Each mouse was sensitized with 5% solution and then challenged with 0.1% solution. The results are compared in Fig.6. It is obvious that the ethyl acetate and acetone solutions

have strong sensitizing ability as compared to the other three with olive oil.

3-5. Comparison of ear thickness in mice sensitized with four kinds of isocyanates in olive oil or DMF:

As the results of the MDI-DMF solution differed unexpectedly from those of the other solutions, TDI, IPDI and HDI were chosen to be tested in DMF for comparison. Likewise, the results in olive oil were compared because of the inertness of olive oil to the skin.

During the sensitizing application, the DMF solution of 5% TDI gave rise to whitish matter which was similar to that seen with MDI-DMF solution at the sensitizing site. Consequently, very mild primary irritation appeared (Fig.7) and almost no increase in ear thickness was obtained upon challenge with 1% solution, while with 5% HDI and 5% IPDI in DMF, primary irritant dermatitis occurred upon sensitization (Figs.8,9). Upon challenge with 1% solution, both isocyanates showed delayed reaction on the ears. These results are compared with those of olive oil in Fig.10. It is apparent that the increase rate with HDI and especially IPDI in DMF is smaller than that with olive oil.

DISCUSSION

The results of this experiment showed that the skin-sensitizing ability of MDI was definitely stronger in the solutions of ethyl acetate, acetone, dichloromethane and toluene than in the olive oil solution.

The reasons why olive oil was used with MDI, though as a suspension, were, firstly, because it is non-irritative to the skin, and secondly, for comparison to the results of the previously reported TDI dermatitis.[9] As the aforementioned four solvents completely dissolve MDI, the results of olive oil suspension cannot be compared with the other results in a strict sense. But the results of TDI and IPDI, which are dissolved completely in olive oil, clearly show that the olive oil solutions have a weaker sensitizing power compared to those of the above four solvents.

When the four solvents are compared with each other, the sensitizing ability in MDI solutions appear to be little different. For TDI, the toluene solution showed stronger power.

In order to discuss the strength of skin-sensitizing ability in detail, percutaneous absorption of the chemical should be considered, as mentioned above. Firstly, the solubility and affinity of the substance within the solution are important, together with its activity coefficient. Another important factor is the partition coefficient between the solvent and the skin barrier layer. Added to these, the physical properties of the solvent itself affect the absorption in relation to the contact time with the skin or cutaneous toxicity.[10] But in the present report, these details were not studied.

In comparing cutaneous toxicity, aprotic polar solvents such as DMF[12] or DMSO[11] are reported to be highly toxic. Persistent damage of the skin has been found in vitro, presumably resulting from displacement of water and removal of lipids. The solutions of such solvents were therefore considered to be strong sensitizers.

In this study, however, each isocyanate in DMF produced weaker or no skin sensitization as compared with those in olive oil. These results indicate that a certain chemical reaction occurred between the isocyanates and the water in DMF solution. Namely, when DMF was used as solvent, the isocyanate groups, which were thought to be necessary for skin sensitization, were consumed by the reaction. Therefore, the skin was not sensitized. Because of the fast reactivity of aromatic isocyanates, the DMF solutions of aromatic isocyanates did not produce skin sensitization at all. But with the other two isocyanates, weak sensitization occurred, possibly due to the slow reactivity of the isocyanate groups.

The reaction of aryl isocyanates with DMF reportedly produces N-aryl-N'-dimethylformamidines, triaryl isocyanates or pentaaryl-1,3,6,8,10-pentazaspiro[4.5]decane-2,4,7,9-tetraones, depending upon the reaction conditions.[13)14)] But in either case, the reaction is known to proceed slowly at room temperature. Such reactions are unlikely to be considered as the main reason for the above results, because skin absorption seems to happen in a matter of minutes.[15)]

The present experimental results are now considered to be caused by the reaction of polyurea formation by the reaction of the isocyanate and the water absorbed by DMF. However, DMF is not an exception with regard to the percentage of water contained in it (max. 0.2%), because acetone (max. 0.25%) and ethyl acetate (max. 0.2%) also contain a considerable amount of water.

Therefore, a trace amount of water originally contained in DMF was not the essential cause of the unusual results. A series of chemical experiments were conducted by Inoue et al.[16)] to clarify the said point. Consequently, it was found that unexpectedly fast moisture absorption of DMF was the cause of polyurea formation resulting from the disappearance of NCO groups in the DMF solution.

Acknowledge :

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Fig.1

Sensitizing site of BALB/c mouse with application of 25% MDI-DMF solution; 3 days after the 1st application

Fig.2

Sensitizing site of BALB/c mouse with application of 25% MDI-DMF solution; 10 days after the 1st application

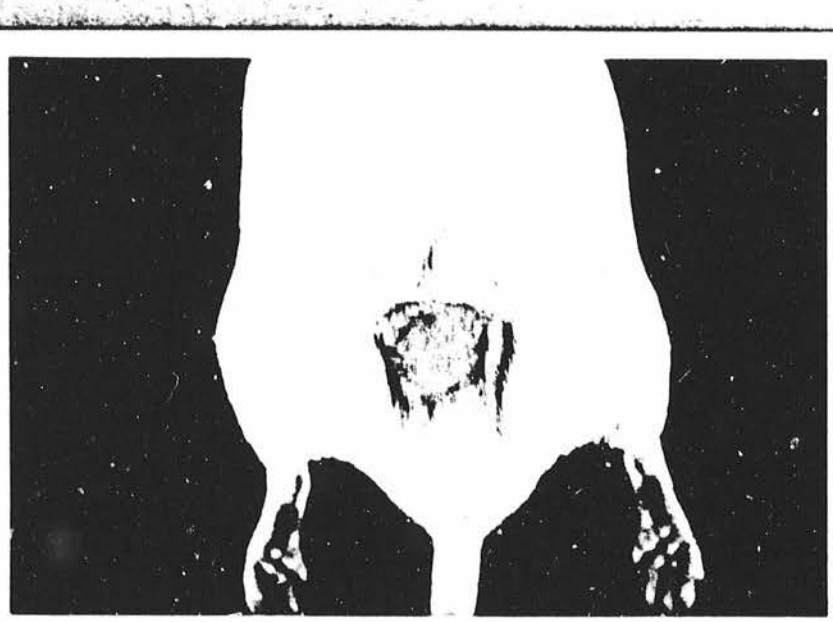


Fig.3

Sensitizing site of BALB/c mouse with application of dimethylformamide (DMF) only; 10 days after the 1st application (Control)

Fig.7

Sensitizing site of BALB/c mouse with application of 5% TDI-DMF solution; 10 days after the 1st application

Fig.8

Sensitizing site of BALB/c mouse with application of 5% HDI-DMF solution; 10 days after the 1st application

Fig.9

Sensitizing site of BALB/c mouse with application of 5% IPDI-DMF solution; 10 days after the 1st application

Fig.4

COMPARISON OF INCREASE RATE IN EAR THICKNESS OF MICE SENSITIZED WITH MDI IN DIFFERENT SOLVENTS (at 48 hr postchallenge; n = 10)

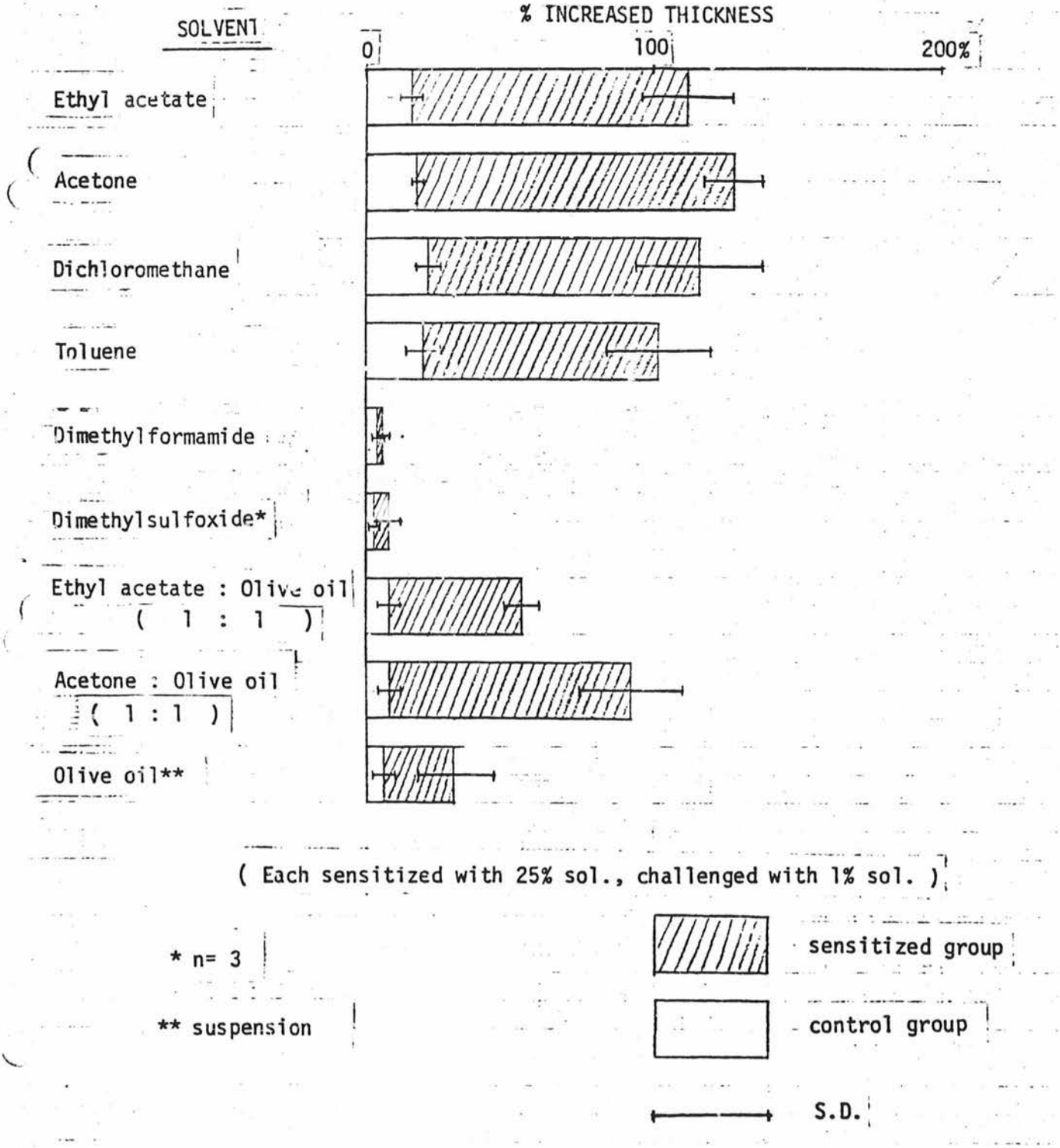
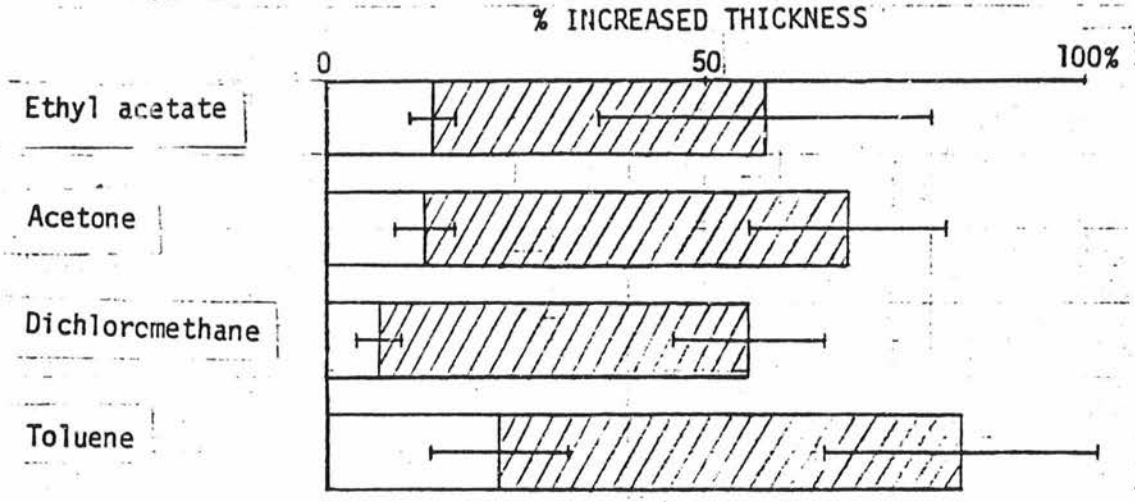


Fig.5

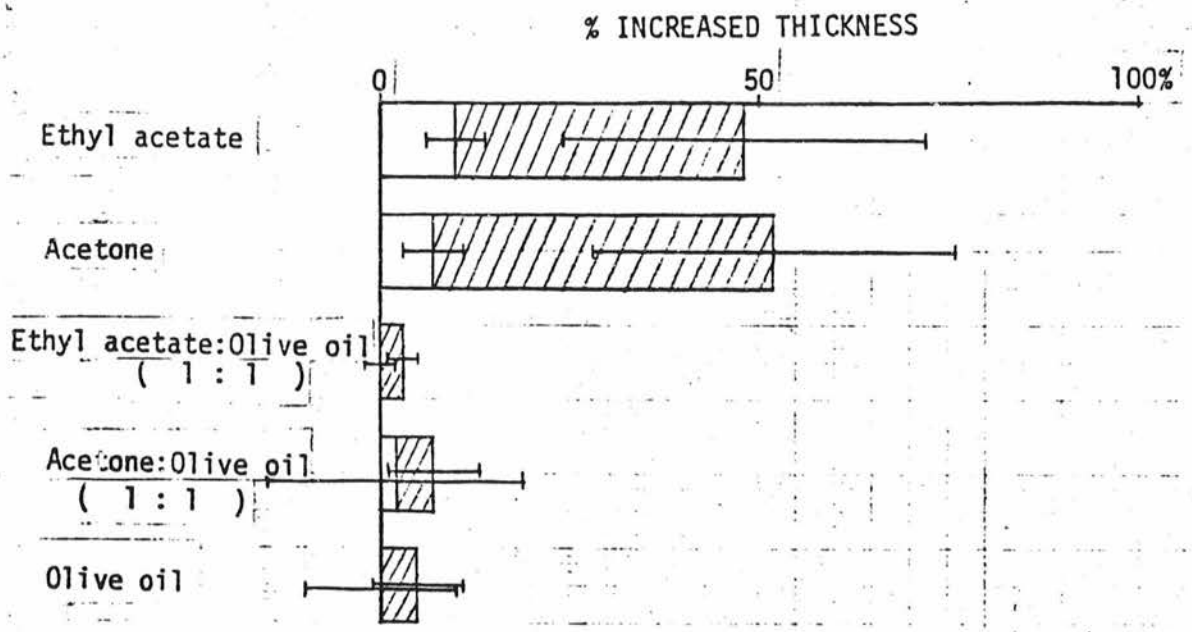
COMPARISON OF INCREASE RATE IN EAR THICKNESS OF MICE SENSITIZED WITH TDI IN DIFFERENT SOLVENTS* (at 48 hr postchallenge; n=5)



* Each sensitized with 5% sol., challenged with 0.3% sol.

Fig.6

COMPARISON OF INCREASE RATE IN EAR THICKNESS OF MICE SENSITIZED WITH IPDI IN DIFFERENT SOLVENTS** (at 48 hr postchallenge; n=3)

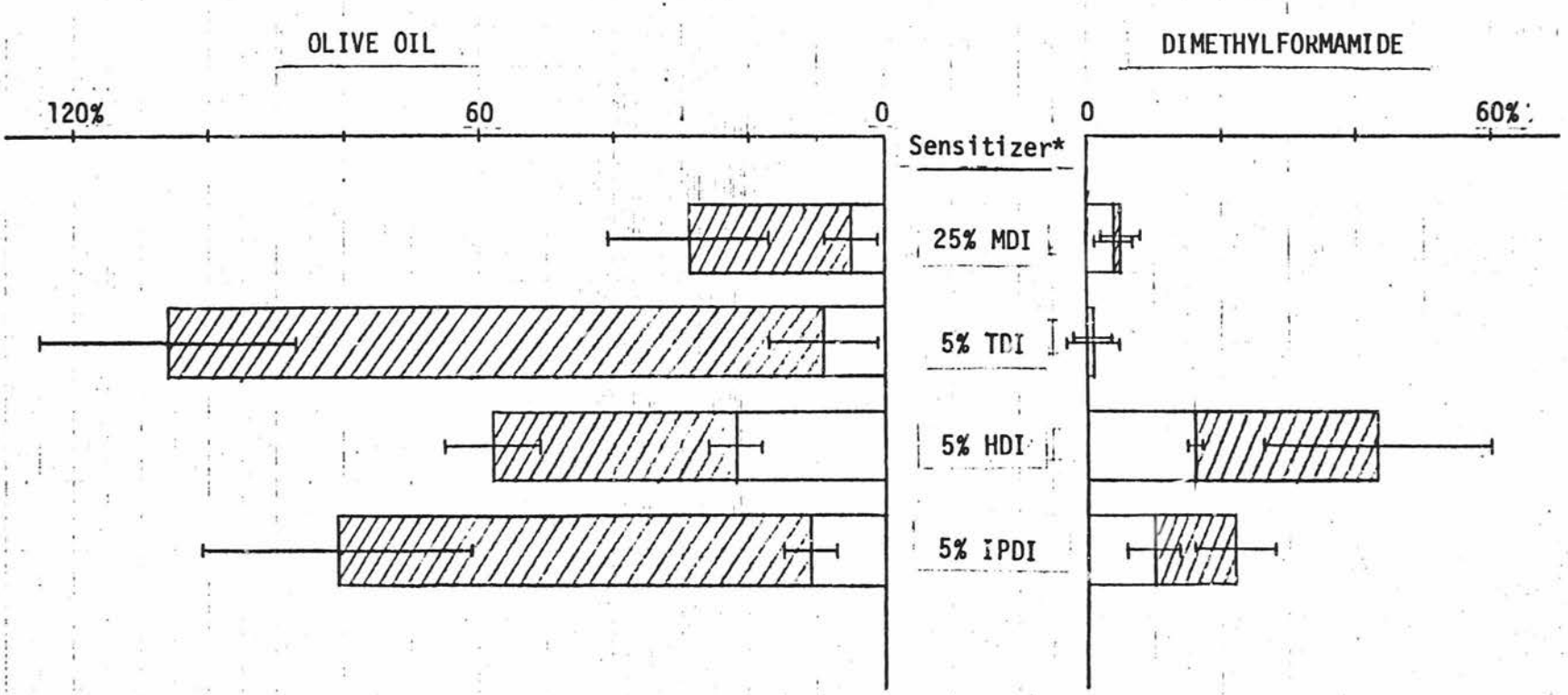


** Each sensitized with 5% sol., challenged with 0.1% sol.

Fig.10

COMPARISON OF INCREASE RATE IN EAR THICKNESS OF MICE SENSITIZED WITH DIFFERENT ISOCYANATES USING OLIVE OIL OR DIMETHYLFORMAMIDE AS SOLVENT

(at 48 hr postchallenge; n=5-10)



* Each challenged with 1% sol.

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